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ELECTROPHORETIC SEPARATION OF BASIC PROTEIN CONTAINING CELLS INFECTED BY DIFFERENT VIRUSES

by

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13. ABSTRACT
Scientists in virusology used electrophoresis to study the synthesis and fraction of total histones in both virus infected cells and the DNP complex as well as examining the dynamics of the accumulation and change of basic proteins in the cells of cytoplasm.

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ENGLISH TITLE: Electrophoretic Separation of Basic Protein Containing Cells Infected by Different Viruses

FOREIGN TITLE: Elektroforeticheskoye Razdelenie Osnovnykh Belkov Kletok, Infitsirovannikh Razlichnymi Virusami

AUTHOR: O. N. Berezina, E. I. Sklyanskaya, I. A. Kozlova, O. P. Peterson, V. M. Zhdanov

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NOTICE

In our experiments we studied changes in the synthesis of total histones and the fraction of this synthesis in the nuclei of infected cells and in the desoxyribonucleprotein (DNP) complex. We also studied the dynamics of accumulation and change in the basic proteins of cytoplasm.

Research was conducted on a 48 hour culture of cells of chicken fibroblasts, cultivated on matrices in medium 199 with the addition of 5% cow serum, and in tissues of eleven day old chicken embryos.

The latter cellular culture was used with the purpose of obtaining a sufficient quantity of the material after preliminarily investigating the accumulation of the studied viruses in it. Chicken embryos were infected with the infectious material introduced into the allantoic cavity in the quantity of $3 \cdot 10^4$ ID_{50} for smallpox vaccine virus, $2 \cdot 10^3$ ID_{50} for grippe virus and $3 \cdot 10^3$ ID_{50} for herpes virus.

On the expiration of a determined period of incubation, the cells obtained from a monolayer and the chicken embryos were homogenized in the hypotonic buffer (0.01 M NaCl; 0.01 M tris pH7.4; 0.015 M $MgCl_2$), while checking the extent of cell destruction by means of microscopic examination of stained smears. The derived homogenate was centrifuged for ten minutes at 1200 rpm to separate the nuclei from the cytoplasmic cell fraction. The nuclei were purified by the modified Penman method (4) using Freon 113 as the detergent. Cytoplasmic cell fraction was repeatedly centrifuged to precipitate the nuclear components. After microscopic control check, an extraction of total acid-soluble proteins of cytoplasm and the purified nuclei was carried out using 0.25 N HCl for 1 1/2 hours. In addition, the basic proteins were fractionized by Johns' first method (1), which was developed for histones. The total histones and their isolated fractions were also extracted from DNP, into 0.9 M NaCl by our modification of Mirsky and Pollister's method.

The DNP compound was characterized by the following data: ratio of protein to DNP = 1.7; Ep = 7200; $E_{min} / E_{max} = 0.6$; $t_{melt} = 93°$; hyperchromic effect = 35%. The separation of basic proteins was carried out by means of electrophoresis in 15% polyacrilamide using McAllister's method (2). The basic proteins of cytoplasm were introduced in the amount of 150 - 200 micrograms per 0.1 ml volume,

and the histones of DNP in the amount of 50 - 100 micrograms. Electrophoresis was carried out in glycine buffer, pH4 using the current of 5 milliamperes for each vial in the course of 1 1/2 - 2 hours. The staining of the protein bands was accomplished using 0.5 - 1% solution of black amide in 7% acetic acid in the course of 1 - 24 hours. Decolorization of the compound was achieved electrophoretically in 7% acetic acid.

Table 1:

Electrophoretic separation of total basic proteins of the cell cytoplasm infected with smallpox vaccine virus (number of bands with different rate of motion)

1 Срок инфекции, час.	2 Основные полосы			3 Минорные полосы		
	4 медленные	5 со средней скоростью	6 быстрые	4 медленные	5 со средней скоростью	6 быстрые
1	1	4	1	3	3	1
3	2	4	1	4	4	1
5	2	8	1	3	1	1
7	1	4	1	4	3	1
9		1	1	3	3	2
7 Контроль		2	1	2	3	1

Key:

1. Term of infection, hours
2. Major bands
3. Minor bands
4. Slow
5. Average rate
6. Fast
7. Control

Analysis of the electrophorograms obtained (Fig. 1, Table 1) indicates the change in the accumulation of basic proteins as a function of the infection time. Already one hour after infection, an increase in the number of protein bands is noticeable, principally in the form of intensely stained protein bands of average electrophoretic mobility. In three hours, additional major bands appear with a slow rate of advance, and toward the fifth hour, the number of major bands exceeds the number found on the non-infected control cells by almost a factor of four, while essentially these are proteins of average electrophoretic mobility. After six hours and particularly after eight hours, a lessening in the number of expressed protein bands is observable. In this same period, the number of minor bands increases.

Analogous results were obtained for basic proteins in the cytoplasm cells of chicken embryos 24 hours after their infection with smallpox vaccine virus (Table 2). Some increase in the number of electrophoretic protein bands also occurred in the case of infection with grippe virus. From the infection with herpes virus there was no particular difference in the number of protein zones, however, their location differed from the control electrophorograms.

Table 2: Electrophoretic separation of total basic proteins of the cell cytoplasm infected with different viruses (number of bands with different rate of motion)

1 Инфицир. вирус	2 Общее число полос	3 Основные полосы			4 Минорные полосы		
		5 медленные	6 со средней скор.	7 быстрые	5 медленные	6 со средней скор.	7 быстрые
8 Осповакцина	20	4	6	2	3	4	1
9 Герпес	12	1	5	1	3	2	1
10 Грипп	15	2	5	1	3	3	1
11 Контроль	10	1	1	1	2	2	1

1. Infectious virus
2. General number of bands
3. Major bands
4. Minor bands
5. Slow
6. Average rate
7. Fast
8. Smallpox vaccine
9. Herpes
10. Grippe
11. Control

The results obtained correspond to the dynamics of the accumulation of viruses in the tissues of chicken embryos for the given period of infection, as it was demonstrated 24 hours after infection, smallpox vaccine virus was detected in titer 10^{-3} ID_{50} and after 48 hours $10^{-5}ID_{50}$; grippe virus correspondingly in titers 10^{-3} and 10^{-7} ID_{50}. Herpes virus failed to be detected after 24 hours, after 48 hours its titer was equal to 10^{-2} but after 72 hours 10^{-4} ID_{50}.

Electrophoretic separation of the basic proteins of cell cytoplasm after 48 hours of infection did not reveal an increase in the number of bands linked to the tinctured infection, but on the contrary it was found to have decreased somewhat in comparison with the control.

Examination of the nuclei of the cells infected with smallpox vaccine virus showed some increase in proteins with average electrophoretic mobility. Separation of the fraction of histones, obtained from DNP and the cytoplasm of cells infected with smallpox vaccine virus preceded investigation of chemical fractionization and electrophoretic differentiation of separate fractions of histones obtained from the DNP of mouse thymuses. These last ones were used by us as standards since in a practical sense the nature of their fraction did not differ from that of the calf's thymus described in the literature.

From the infected tissues, just as from the thymuses of mice, four fractions of histones were separated: lysin-rich histones - f1, relatively lysin-rich fractions f2a and f2b and ones rich in arginine - f3.

The data of the electrophoretic divisions of separate fractions of DNP histones is evidence that in cells infected with viruses of smallpox vaccine and grippe a gradual disappearance of lysin-rich fractions f1 take place, at the same time that in fractions moderately rich in lysine with an average rate of electrophoresis(f2a and f2b) a supplementary number of major and minor bands appear. The fractions rich in arginine, with infected material do not differ from the control. (Table 3, Fig. 3)

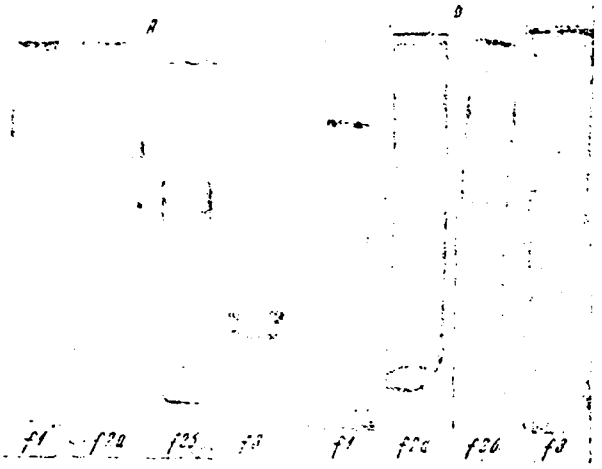

Figure 3: Histone fractions of DNP in chicken embryos.
A - control, B - experiment

Table 3: Electrophoretic separation of histone fractions in DNP cells infected with different viruses.*

1 Инфицирующий вирус	f1		f2a		f2b		f3	
	2 осн.	3 мин.	2 осн.	3 мин.	2 осн.	3 мин.	1 осн.	3 мин.
4 Оспа	1	1	2	3	4	2	2	
5 Герпес	2	1	1—2	2	1—2		2	
6 Грипп		1	3	2	1—2		1	
7 Контроль	1—2	1		2			2	

*Here and in table 4 - principal and minor refer to bands

Table 3(cont.)
Key:
1. Infectious virus
2. Major
3. Minor
4. Smallpox
5. Herpes
6. Grippe
7. Control

It proved possible to separate the basic cytoplasmic proteins into four fractions (Fig. 4, Table 4) which correspond to the four histone fractions in terms of their electrophoretic mobility and solubility in various solvents. As is evident from Table 4 and Figure 4, within 24 hours after infection with smallpox vaccine virus in the fractions corresponding to ones moderately rich in lysin (f2a and f2b), a significant increase in the number of bands is noticeable during the periods of infection studied.

The data obtained makes it possible to explain the observed increase in the amount of total basic proteins in cytoplasm. As can be seen from the preceding results, this increase takes place in the form of fractions, corresponding to fractions of histones with moderate lysin content (f2a and f2b). It may be surmised that the inhibiting activity of basic proteins of cytoplasm observed by us is probably linked with precisely this fraction of basic proteins. The increase in histone fractions, relatively rich in lysin (f2a and f2b) in the DNP of infected cells also serves to support the hypothesis set forth since the possibllity is not excluded that these proteins are synthesized in cytoplasm and transferred into the nuclear part of the cells.

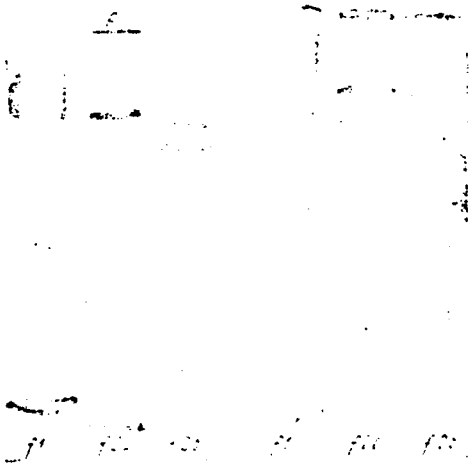

Figure 4. Basic proteins in cytoplasm cells of chicken embryos. A.- control, B - experiment

However, the final conclusion to this question may be drawn only after studying the biological activity of the individual fractions.

In our view, the essential fact is also the disclosure of a decrease in lysin-rich fractions (f1) in DNP and in the cytoplasm of infected cells. Basically, the mechanism of this phenomenon may be either an

increase in the labile state of the bonds connecting DNA (deoxyribonucleic acid) and protein or else a change in the properties of the proteins themselves.

Table 4: Electrophoretic separation of basic proteins in cytoplasm cells, infected with smallpox vaccine virus

1 Сроки инфекции, день	f1		f2a		f2b		f3	
	2 осн.	3 мин.	2 осн.	3 мин.	2 осн.	3 мин.	2 осн.	3 мин.
1	1		5	5	2	1	2	
2	1		1	2—3	5	3	2	
4 Контр.	2	1	1	4—5	1--2		2	

Key:

1. Periods of infection, day
2. Major
3. Minor
4. Control

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